REMARKS

Status of the Claims

Claims 1, 12, 14, 15, 17, 18, 20, 21, 23, and 24 are pending in the present application. Claims 2-11, 13, 16, 19, 22, and 25 were previously canceled. Claim 1 is amended to clarify that the efficiency of gene transfer is increased in comparison to the efficiency of gene transfer by *Agrobacterium*, wherein the plant cells or plant tissues are not heated or centrifuged according to steps a) and b). No new matter is entered by way of this amendment. Reconsideration is respectfully requested.

Substance of the Interview

Applicants and Applicants' representative wish to thank the Examiner for extending the courtesy of an interview on March 12, 2009. The issues discussed during the interview are essentially those that were described in the interview summary issued on March 20, 2009. Applicants further responses to the Examiner's comments and the issues raised during the interview are discussed herein.

Issues Under 35 U.S.C. § 112, Second Paragraph

Claims 1, 12, 14, 15, 17, 18, 20, 21, 23, and 24 are rejected under 35 U.S.C. § 112, second paragraph, as allegedly indefinite. Specifically, the Examiner states that the phrase "promoting efficiency" implies there is an increase in gene transfer efficiency, but it is unclear to what the increase is compared, *see* Office Action, page 3.

Claim 1 is amended to specify a method for promoting efficiency of gene transfer into plant embryos, plant calli or cultured plant cells by a bacterium belonging to genus *Agrobacterium*, which comprises: a) heating and centrifuging said plant embryos, plant calli or cultured plant cells; and b) contacting said plant cells or plant tissue with the bacterium so that the gene is transferred into the plant; wherein contact between the plant cells or plant tissue and the bacterium occurs after or while heating and/or centrifuging the plant cells or plant tissue, wherein heating is performed at a temperature of 37 °C to 52 °C for 1 minute to 24 hours, wherein said centrifuging is carried out under a centrifugal acceleration of 1000G to 150,000G

for 1 second to 4 hours and wherein the efficiency of gene transfer is increased in comparison to the efficiency of gene transfer by *Agrobacterium*, wherein the plant cells or plant tissues are not heated or centrifuged according to steps a) and b).

As amended, claim 1 describes that the increase in gene transfer efficiency of the described plant embryos, calli or cultured cells is compared to those cells or plant tissues that are not heated or centrifuged as described in steps a) and b). Accordingly, the claims clearly describe that the increase in gene transfer efficiency is compared to plant samples that have not been heated and centrifuged. Based upon the foregoing, the claims are not indefinite and Applicants respectfully request withdrawal of the rejection.

Issues Under 35 U.S.C. § 112, First Paragraph, Enablement

Claims 1, 12, 14, 15, 17, 18, 20, 21, 23, and 24 are rejected under 35 U.S.C. § 112, first paragraph, as allegedly lacking enablement. Applicants respectfully traverse.

During the interview of March 12, 2009, the Examiner and Applicants' representative further discussed the enablement rejection. In particular, the Examiner and Applicants' representative discussed enablement of the temperature range, the time period for incubation, the range of centrifuge acceleration, and the time periods for centrifugation, as specified in the instant claims. For the reasons set forth below, Applicants submit that the application provides adequate enablement support for each of the above-described elements.

Temperature Range

The pending claims specify that the described plant samples are incubated at a temperature ranging from 37°C to 52°C. Applicants submit that Experiment 2 in the Hiei Declaration, which Applicants submitted to the Office on October 24, 2008, clearly demonstrates that transformation efficiency is promoted across the entire range of temperatures specified in the instant claims. Applicants understand that the Examiner believes that the October 24, 2008, Hiei Declaration demonstrates enablement of the temperature range and that this aspect of the enablement rejection is overcome, *see* Interview Summary, March 20, 2009, ("Interview Summary").

IncubationTime

The Examiner further stated during the interview that the present application does not support a time period for incubation ranging from 1 minute to 24 hours, *see* Interview Summary. The Examiner stated that she did not believe that such a broad range of time periods was allowable, since the data of record only describes incubation periods of up to two hours. However, the Examiner also stated that this aspect of the rejection could be overcome if Applicants would provide data showing that the claimed method could be used with plant samples that were incubated for *e.g.*, 10 hours and 20 hours, at a temperature within the range specified in the instant claims.

In an effort to expedite prosecution, Applicants submit herewith a Declaration under 37 CFR 1.132 (Hiei Declaration I), which supports the enablement of incubation time periods specified in the instant claims. The Hiei Declaration I describes immature rice embryos that were incubated in a water bath at various temperatures before infection with *A. tumefaciens*. The Declaration describes an increase in gene transfer efficiency by heat treatment at 37° for 12 hours or 24 hours using immature embryos of IR64, *see* Figure 1. Similar effects are also shown in the tests using the variety Yukihikari, wherein the heating period was 10 hours or 20 hours, *see* Figure 3.

Based upon the foregoing, Applicants submit that the claimed methods may be used with plants samples that are incubated across the temperatures and time periods specified in the instant claims. Accordingly, Applicants submit that this aspect of the rejection is overcome.

Centrifugation Acceleration Range

The Examiner stated during the interview that she did not believe that the centrifugation speed range, *i.e.*, from 1000G to 150,000G, was supported in the present application. In response, Applicants' representative presented the centrifugation data from co-pending U.S. Application No. 10/089,696, which the Examiner stated was sufficient to overcome this aspect of the rejection, *see* Interview Summary. Submitted herewith is the centrifugation data Applicants' representative presented to the Examiner during the interview, *see* Exhibit A, *i.e.*, U.S. Application No. 10/089,696, *see*, in particular, Examples and Table 6. Accordingly, this aspect of the enablement rejection is overcome.

Time Period of Centrifugation

During the interview, the Examiner stated that only a portion of the time period of the centrifugation range was enabled, *i.e.,* 1 second to one hour, *see* Hiei Declaration submitted to the Office on October 24, 2008. The Examiner asserted that further data is required to support the time range of centrifugal acceleration for the longer periods of centrifugation. According to the Examiner, this aspect of the rejection may be overcome if Applicants provide data demonstrating longer periods of acceleration, *e.g.*, at four hours.

In an effort to expedite prosecution, Applicants submit herewith a Declaration under 37 CFR 1.132 (Hiei Declaration II), which supports the enablement of the time period for centrifugation specified in the instant claims. The Hiei Declaration II describes an increase in gene transfer efficiency of immature rice embryos that were subjected to centrifugation treatment at 4 hours, *see* Table 1 and Figure 2.

Based upon the foregoing, Applicants submit that the claimed method may be used with plants samples that are subjected to centrifugation across the centrifugation time periods specified in the instant claims. Accordingly, Applicants submit that this aspect of the rejection is overcome.

Based upon the foregoing, Applicants submit that the present application supports the instant claims. Accordingly, withdrawal of the rejection under 35 U.S.C. § 112, first paragraph enablement is requested.

Issues Under 35 U.S.C. § 103(a)

Claims 1, 12, 14, 15, 17, 18, 20, 21, 23, and 24 are rejected under 35 U.S.C. § 103(a) as allegedly obvious over PCT Publication No. WO 98/54961 to Hansen in view of Lyznik *et al.*, *The Plant Journal*, 1995, 8:177-186. Specifically, the Examiner states that Hansen teaches a method of gene transfer to maize *Agrobacterium* by heating maize calli at 45°C for 4 minutes, *see* Office Action, page 7. The Examiner further states that Hansen does not teach centrifugation from 1000G to 150,000G, *see* Office Action, page 8. According to the Examiner, Lyznik teaches that maize protoplasts are harvested in Eppendorf tubes by centrifugation for 5-10 seconds at

1000G to replace medium with extraction buffer, *see* Office Action, page 8. The Examiner further asserts that a skilled artisan would have been motivated to modify the method taught by Hansen to centrifuge the cells to facilitate changing the buffer medium, *see* Office Action, page 8. In response to Applicants previous arguments, the Examiner agrees that Applicants demonstrated unexpected results, *see* Office Action, page 8. However, the Examiner further asserts that that the claims are not commensurate in scope with the unexpected results, *see* Office Action, pages 8-9.

During the interview, the Examiner and Applicants' representative discussed Example 2 of the present application, which describes unexpected results for maize embryos that were treated at 46°C for 3 minutes and centrifuged at 20KG for three minutes, *i.e.*, GUS expression is increased in comparison to heat or centrifugation treatment alone. As noted above, the Examiner agreed that the temperature and time were close enough to be considered comparable to conditions described for the maize calli discussed in Hansen. However, the Examiner stated that she needed a reason to believe that the results from an immature embryo, as described by Applicants in Example 2 of the present application, can be extended to the heat-treated calli, described in Hansen, in order to overcome this particular rejection by relying on unexpected results.

The unexpected results relied on by Applicants use the same species, tissue-type and a comparable incubation period and incubation temperature to those described in Hansen.

Applicants submit that the results for maize embryos described in Example 2 of the present application are directly comparable to the heat-treated maize tissue discussed in Hansen. Hansen teaches heat shock treatment of immature maize embryos in addition to the calli discussed by the Examiner. On page 19, paragraph 1, Hansen teaches that maize tissues are heated shocked for 4 minutes at 45°C. On page 19 at paragraph 5, Hansen states that "[e]mbryos and Type I callus are heat-shocked and then inoculated with Agrobacterium." Page 21, first paragraph of Hansen, states that "[e]mbryos from various maize lines are inoculated with Agrobacterium…with or without a heat shock pre-treatment", *see* also Table 6. As noted above, the Examiner agreed that the temperatures and incubation periods described in Example 2 of the

present application are comparable to Hansen. Based upon the foregoing, Applicants submit that the instant claims are not obvious in view of Hansen and Lyznik. Withdrawal of the rejection is respectfully requested.

The unexpected results are commensurate in scope with the instant claims.

The Examiner further stated during the interview that if Applicants are able to overcome the rejection under 35 U.S.C. § 103(a), the Examiner will continue to search the art for other tissues, temperatures and incubation times to ensure that the entire breadth of the claims is truly free of the art. The Examiner further stated that part of the problem is that the unexpected results relied upon to overcome the art are only achieved with certain combinations of incubation times and temperatures. According to the Examiner, some of the times and temperatures do not produce the unexpected result based upon the data of record. The Examiner further asserted that in order to overcome the obviousness rejection based upon unexpected results, the claims need to be commensurate in scope with the unexpected results, *see* Interview Summary.

The nonobviousness of a broader claimed range can be supported by evidence based on unexpected results from testing a narrower range if one of ordinary skill in the art would be able to determine a trend in the exemplified data which would allow the artisan to reasonably extend the probative value thereof. *In re Kollman*, 595 F.2d 48, 201 USPQ 193 (CCPA 1979)

Applicants submit that the novel, non-obvious methods as claimed herein and described in the present application are based upon the unexpected finding that gene transfer efficiency can be promoted by performing heat treatment and centrifugation on plant tissues. Applicants have performed many experiments and provided actual working examples under various temperatures and time periods commensurate in scope with the instant claims. Applicants have further submitted a plurality of Declarations under 37 C.F.R. § 1.132, which further supports the advantageous and unexpected effects of the claimed methods. Accordingly, Applicants submit that the unexpected effects have been demonstrated across the breadth of the claims. Applicants further submit that it is impractical to claim or exemplify all combinations of conditions by which the effect of the present invention is obtained. Applicants further submit that narrowing the claims is unreasonable and not necessary. Further, as amended, the claims only encompass

those methods wherein an increase in gene transfer efficiency is achieved *via* the described treatment. That is, those embodiments wherein the promotion of gene transfer efficiency is not attained is excluded from the scope of the instant claims. In addition, Applicants submit that one of ordinary skill in the art would be able to determine a trend in the provided data which would allow the artisan to reasonably extend the probative value thereof.

Based upon the foregoing, Applicants submit that the claims are in condition for allowance.

CONCLUSION

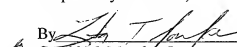
In view of the above amendment and remarks, Applicants believes the pending application is in condition for allowance.

Should there be any outstanding matters that need to be resolved in the present application, the Examiner is respectfully requested to contact L. Parker, Reg. No. 46,046, at the telephone number of the undersigned below, to conduct an interview in an effort to expedite prosecution in connection with the present application.

If necessary, the Commissioner is hereby authorized in this, concurrent, and future replies to charge payment or credit any overpayment to Deposit Account No. 02-2448 for any additional fees required under 37.C.F.R. §§1.16 or 1.17; particularly, extension of time fees.

Dated: June 15, 2009

Respectfully submitted,

By 
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